

SEQUENCE LISTING

<110> SHERMAN, LINDA A. LUSTGARTEN, JOSEPH <120> RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS <130> 48340/55793 <140> 08/812,393 <141> 1997-03-05 <150> 60/012,845 <151> 1996-03-05 <160> 64 <170> PatentIn Ver. 2.1 <210> 1 <211> 1350 <212> DNA <213> Artificial Sequence <220> <221> CDS <222> (1)..(1332) <223> Description of Artificial Sequence: Synthetic single chain TCR derivative nucleotide sequence <400> 1 ctc gag atg cag agg aac ctg gga gct gtg ctg ggg att ctg tgg gtg Leu Glu Met Gln Arg Asn Leu Gly Ala Val Leu Gly Ile Leu Trp Val 48 10 cag att tgc tgg ctg aaa gaa cag caa gtg cag cag agt ccc gca tcc 96 Gln Ile Cys Trp Leu Lys Glu Gln Gln Val Gln Gln Ser Pro Ala Ser ttg gtt ctg cag gag ggg gag aac gca gag ctc cag tgt agc ttt tcc Leu Val Leu Gln Glu Gly Glu Asn Ala Glu Leu Gln Cys Ser Phe Ser atc ttt aca aac cag gtg cag tgg ttt tac caa cgt cct ggg gga aga Ile Phe Thr Asn Gln Val Gln Trp Phe Tyr Gln Arg Pro Gly Gly Arg ctc gtc agc ctg ttg tac aat cct tct ggg aca aag cag agt ggg aga 240 Leu Val Ser Leu Leu Tyr Asn Pro Ser Gly Thr Lys Gln Ser Gly Arg 70 ctg aca tcc aca aca gtc att aaa gaa cgt cgc agc tct ttg cac att Leu Thr Ser Thr Thr Val Ile Lys Glu Arg Arg Ser Ser Leu His Ile 90 85

| | | | | | | | | | | | ctc Leu | | | | | 336 |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------------------|-------------------|-------------------|--------------------|-------------------|-------------------|-----|
| tct Ser | gga Gly | gga Gly 115 | agc Ser | aat Asn | gca Ala | aag Lys | cta Leu 120 | acc Thr | ttc Phe | gly aaa | aaa Lys | ggc Gly 125 | act Thr | aaa Lys | ctc Leu | 384 |
| tct Ser | gtt Val 130 | aaa Lys | tca Ser | ggt Gly | ggc Gly | gga Gly 135 | gly ggg | tct Ser | ggc Gly | Gly aaa | ggt Gly 140 | gga Gly | tcc Ser | gly aaa | ggt Gly | 432 |
| gga Gly 145 | ggc Gly | tca Ser | gag Glu | gct Ala | gca Ala 150 | gtc Val | acc Thr | caa Gln | agc Ser | cca Pro 155 | aga Arg | aac Asn | aag Lys | gtg Val | gca Ala 160 | 480 |
| gta Val | aca Thr | gga Gly | gga Gly | aag Lys 165 | gtg Val | aca Thr | ttg Leu | agc Ser | tgt Cys 170 | aat Asn | cag Gln | act Thr | aat Asn | aac Asn 175 | cac His | 528 |
| aac Asn | aac Asn | atg Met | tac Tyr 180 | tgg Trp | tat Tyr | cgg Arg | cag Gln | gac Asp 185 | acg Thr | Glà aaa | cat His | Gly aaa | ctg Leu 190 | agg Arg | ctg Leu | 576 |
| atc Ile | cat His | tat Tyr 195 | tca Ser | tat Tyr | ggt Gly | gct Ala | ggc Gly 200 | agc Ser | act Thr | gag Glu | aaa Lys | gga Gly 205 | gat Asp | atc Ile | cct Pro | 624 |
| gat Asp | gga Gly 210 | tac Tyr | aag Lys | gcc Ala | tcc Ser | aga Arg 215 | cca Pro | agc Ser | caa Gln | gag Glu | aac Asn 220 | ttc Phe | tcc Ser | ctc Leu | att Ile | 672 |
| ctg Leu 225 | gag Glu | ttg Leu | gct Ala | acc Thr | ccc Pro 230 | tct Ser | cag Gln | aca Thr | tca Ser | gtg Val 235 | tac Tyr | ttc Phe | tgt Cys | gcc Ala | agc Ser 240 | 720 |
| ggt Gly | gag Glu | aca Thr | G1 Y 999 | acc Thr 245 | aac Asn | gaa Glu | aga Arg | tta Leu | ttt Phe 250 | Phe | ggt Gly | cat His | gga Gly | acc Thr 255 | aag Lys | 768 |
| ctg Leu | tct Ser | gtc Val | ctg Leu 260 | Thr | agt Ser | aac Asn | tcc Ser | atc Ile 265 | Met | tac Tyr | ttc Phe | agc Ser | cac His 270 | Phe | gtg Val | 816 |
| ccg Pro | gtc Val | Phe 275 | Leu | cca Pro | gcg Ala | aag Lys | Pro 280 | Thr | acg Thr | acg Thr | cca Pro | gcg Ala 285 | Pro | g cga Arg | cca Pro | 864 |
| cca Pro | aca Thr 290 | Pro | gcg Ala | ccc Pro | acc Thr | atc 11e 295 | e Ala | tcg Ser | cag Glr | g cco n Pro | ctg Leu 300 | Ser | ct <u>c</u> Lev | g cgc | cca Pro | 912 |
| tct Ser 305 | Ser | tct Ser | aga Arg | gat J Asp | ccc Pro 310 | Lys | teto Lev | tgo LCys | tac Tyi | c cto Let 315 | ı Lev | gat Asp | gga Gly | a ato | ctc Leu 320 | 960 |

| | | | | | | | | | | | | aga Arg | | | | 1008 |
|-------------------|----------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| | | | | | | | | | | | | cag Gln | | | | 1056 |
| tat Tyr | aac Asn | gag Glu 355 | ctc Leu | aat Asn | cta Leu | gga Gly | cga Arg 360 | aga Arg | gag Glu | gag Glu | tac Tyr | gat Asp 365 | gtt Val | ttg Leu | gac Asp | 1104 |
| aag Lys | aga Arg 370 | cgt Arg | ggc Gly | cgg Arg | gac Asp | cct Pro 375 | gag Glu | atg Met | GJA aaa | gga Gly | aag Lys 380 | ccg Pro | aga Arg | agg Arg | aag Lys | 1152 |
| aac Asn 385 | cct Pro | cag Gln | gaa Glu | ggc Gly | ctg Leu 390 | tac Tyr | aat Asn | gaa Glu | ctg Leu | cag Gln 395 | aaa Lys | gat Asp | aag Lys | atg Met | gcg Ala 400 | 1200 |
| gag Glu | gcc Ala | tac Tyr | agt Ser | gag Glu 405 | att Ile | Gly 999 | atg Met | aaa Lys | ggc Gly 410 | gag Glu | cgc Arg | cgg Arg | agg Arg | ggc Gly 415 | aag Lys | 1248 |
| Gly aaa | cac His | gat Asp | ggc Gly 420 | ctt Leu | tac Tyr | cag Gln | ggt Gly | ctc Leu 425 | agt Ser | aca Thr | gcc Ala | acc Thr | aag Lys 430 | gac Asp | acc Thr | 1296 |
| tac Tyr | gac Asp | gcc Ala 435 | Leu | cac His | atg Met | cag Gln | gcc Ala 440 | ctg Leu | ccc Pro | cct Pro | cgc Arg | taa | gcg | gcc | gcc | 1344 |
| acc | gcg | | | | | | | | | | | | | | | 1350 |
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| <40 Leu 1 | | Met | : Glr | Arg | | Leu | Gly | Ala | Val | | Gly | ' Ile | Leu | Trp | Val | |
| Glr | Ile | : Суа | Trp 20 | | Lys | Glu | Glr | Gln 25 | | Gln | Gln | Ser | Pro 30 | Ala | Ser | |
| Leu | ı Val | . Leu | | ı Glu | ı Gly | Glu | Asr 40 | | Glu | Lev | Glr | 1 Cys | | Phe | e Ser | |
| Ile | Phe 50 | | Asr | n Glr | ı Val | . Glr 55 | | Phe | Tyr | Glr | Arc 60 | | Gly | gly | / Arg | |

- Leu Val Ser Leu Leu Tyr Asn Pro Ser Gly Thr Lys Gln Ser Gly Arg
 65 70 75 80
- Leu Thr Ser Thr Thr Val Ile Lys Glu Arg Arg Ser Ser Leu His Ile 85 90 95
- Ser Ser Ser Gln Ile Thr Asp Ser Gly Thr Tyr Leu Cys Ala Ser Asn 100 105 110
- Ser Gly Gly Ser Asn Ala Lys Leu Thr Phe Gly Lys Gly Thr Lys Leu 115 120 125
- Ser Val Lys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly 130 135 140
- Gly Gly Ser Glu Ala Ala Val Thr Gln Ser Pro Arg Asn Lys Val Ala 145 150 155 160
- Val Thr Gly Gly Lys Val Thr Leu Ser Cys Asn Gln Thr Asn Asn His
 165 170 175
- Asn Asn Met Tyr Trp Tyr Arg Gln Asp Thr Gly His Gly Leu Arg Leu 180 185 190
- Ile His Tyr Ser Tyr Gly Ala Gly Ser Thr Glu Lys Gly Asp Ile Pro 195 200 205
- Asp Gly Tyr Lys Ala Ser Arg Pro Ser Gln Glu Asn Phe Ser Leu Ile 210 215 220
- Leu Glu Leu Ala Thr Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser 225 230 235 240
- Gly Glu Thr Gly Thr Asn Glu Arg Leu Phe Phe Gly His Gly Thr Lys 245 250 255
- Leu Ser Val Leu Thr Ser Asn Ser Ile Met Tyr Phe Ser His Phe Val 260 265 270
- Pro Val Phe Leu Pro Ala Lys Pro Thr Thr Thr Pro Ala Pro Arg Pro 275 280 285
- Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro 290 295 300
- Ser Ser Ser Arg Asp Pro Lys Leu Cys Tyr Leu Leu Asp Gly Ile Leu 305 310 315
- Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu Arg Val Lys Phe 325 330 335
- Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu 340 345 350
- Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp 355 360 365

| 370 375 380 | , nys |
|--|------------|
| Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met 385 390 395 | Ala 400 |
| Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Gly 405 410 415 | |
| Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp 420 425 430 | Thr |
| Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg 435 440 | |
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<213> Artificial Sequence

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|--|--------|
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| <213> Artificial Sequence | |
| <220> | |
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| <212> DNA | |
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| <220> | |
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| ggttcctctt cagggtccag aatatgt | 27 |
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| 12137 ALCILICIAL DOQUENCE | |
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| -211 - 20 | |

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| tgg gtg cag agc cag cag aag gtg cag cag agc cca gaa tcc ctc agt Trp Val Gln Ser Gln Gln Lys Val Gln Gln Ser Pro Glu Ser Leu Ser 20 25 30 | 96 |

| _ | | | | | _ | _ | | | | _ | | tca Ser 45 | - | | | 144 |
|--|---|--|--------------------------------|------------------------|------------------------|----------------|-------------------------|-------------------------|-------------------------|--------------------------------|----------------|-------------------------|-------------------------|-------------------|------------------------|-----|
| | | | | | | | | | | | | gga Gly | | | | 192 |
| | | | | | | | | | | | | aaa Lys | | | | 240 |
| ttc Phe | aca Thr | gct Ala | cac His | ctc Leu 85 | aat Asn | aag Lys | gcc Ala | agc Ser | ctg Leu 90 | cat His | gtt Val | tcc Ser | ctg Leu | cac His 95 | atc Ile | 288 |
| | | | | | | | | | | | | tgt Cys | | | | 336 |
| gat Asp | tat Tyr | aac Asn 115 | cag Gln | Gly aaa | aag Lys | ctt Leu | atc Ile 120 | ttt Phe | G1 y 999 | cag Gln | ggt Gly | acc Thr 125 | aag Lys | tta Leu | tct Ser | 384 |
| | aag Lys 130 | | | | | | | | | | | | | | | 393 |
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| <21: <21: <21: <40: Met | 1> 1: 2> P: 3> H: 0> 4 Lys | 31 RT omo 4 Ser | Leu | Ser 5 | | | | | 10 | | | Leu Glu | | 15 | | |
| <21: <21: <21: <40: Met 1 | 1> 1: 2> P: 3> H: 0> 4 Lys | 31 RT omo 4 Ser Gln | Leu Ser 20 | Ser 5 Gln | Gln | Lys | Val | Gln 25 | 10 Gln | Ser | Pro | Glu | Ser 30 | 15 Leu | | |
| <21: <21: <21: <40: Met 1 Trp | 1> 1. 2> P: 3> H 0> 4 Lys Val | 31 RT OMO 4 Ser Gln Glu 35 | Leu Ser 20 | Ser 5 Gln Gly | Gln Met | Lys Ala | Val Ser 40 | Gln 25 Leu | 10 Gln Asn | Ser Cys | Pro Thr | Glu Ser 45 Gly | Ser 30 Ser | 15 Leu Asp | Ser | |
| <21: <21: <21: <40: Met 1 Trp Val | 1 > 1. 2 > P. 3 > H. 0 > 4 Lys Val Pro Phe 50 | 31 RT omo 4 Ser Gln Glu 35 | Leu Ser 20 Gly | Ser 5 Gln Gly | Gln Met Trp | Lys Ala Trp 55 | Val Ser 40 | Gln 25 Leu Arg | 10 Gln Asn Gln | Ser Cys His | Pro Thr Ser 60 | Glu Ser 45 | Ser 30 Ser Glu | Leu Asp Gly | Ser Arg | |
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Asp Tyr Asn Gln Gly Lys Leu Ile Phe Gly Gln Gly Thr Lys Leu Ser

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130

Thr Arg Leu Thr Val Ser

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<212> PRT
<213> Homo sapiens
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His Met Glu Ala Ala Val Thr Gln Ser Pro Arg Ser Lys Val Ala Val 20 25 30

Thr Gly Gly Lys Val Thr Leu Ser Cys His Gln Thr Asn Asn His Asp 35 40 45

Tyr Met Tyr Trp Tyr Arg Gln Asp Thr Gly His Gly Leu Arg Leu Ile 50 55 60

His Tyr Ser Tyr Val Ala Asp Ser Thr Glu Lys Gly Asp Ile Pro Asp 65 70 75 80

Gly Tyr Lys Ala Ser Arg Pro Ser Gln Glu Asn Phe Ser Leu Ile Leu 85 90 95

Glu Leu Ala Ser Leu Ser Gln Ser Ala Val Tyr Phe Cys Ala Ser Ser 100 105 110

Asp Phe Ala Gly Thr Gly Gly Phe Tyr Glu Gln Tyr Phe Gly Pro Gly 115 120 125

Thr Arg Leu Thr Val Ser 130

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic peptide

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SEQUENCE LISTING

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| tat Tyr | aac Asn | gag Glu 355 | ctc Leu | aat Asn | cta Leu | gga Gly | cga Arg 360 | aga Arg | gag Glu | gag Glu | tac Tyr | gat Asp 365 | gtt Val | ttg Leu | gac Asp | 1104 |
| aag Lys | aga Arg 370 | cgt Arg | ggc Gly | cgg Arg | gac Asp | cct Pro 375 | gag Glu | atg Met | gjå aaa | gga Gly | aag Lys 380 | ccg Pro | aga Arg | agg Arg | aag Lys | 1152 |
| aac Asn 385 | cct Pro | cag Gln | gaa Glu | ggc Gly | ctg Leu 390 | tac Tyr | aat Asn | gaa Glu | ctg Leu | cag Gln 395 | aaa Lys | gat Asp | aag Lys | atg Met | gcg Ala 400 | 1200 |
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 65 70 75 80
- Leu Thr Ser Thr Thr Val Ile Lys Glu Arg Arg Ser Ser Leu His Ile 85 90 95
- Ser Ser Ser Gln Ile Thr Asp Ser Gly Thr Tyr Leu Cys Ala Ser Asn 100 . 105 110
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- Ser Val Lys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly 130 135 140
- Gly Gly Ser Glu Ala Ala Val Thr Gln Ser Pro Arg Asn Lys Val Ala 145 150 155 160
- Val Thr Gly Gly Lys Val Thr Leu Ser Cys Asn Gln Thr Asn Asn His
- Asn Asn Met Tyr Trp Tyr Arg Gln Asp Thr Gly His Gly Leu Arg Leu 180 185 190
- Ile His Tyr Ser Tyr Gly Ala Gly Ser Thr Glu Lys Gly Asp Ile Pro 195 200 205
- Asp Gly Tyr Lys Ala Ser Arg Pro Ser Gln Glu Asn Phe Ser Leu Ile 210 215 220
- Leu Glu Leu Ala Thr Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser 225 230 235 240
- Gly Glu Thr Gly Thr Asn Glu Arg Leu Phe Phe Gly His Gly Thr Lys 245 250 255
- Leu Ser Val Leu Thr Ser Asn Ser Ile Met Tyr Phe Ser His Phe Val 260 265 270
- Pro Val Phe Leu Pro Ala Lys Pro Thr Thr Pro Ala Pro Arg Pro 275 280 285
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- Ser Ser Ser Arg Asp Pro Lys Leu Cys Tyr Leu Leu Asp Gly Ile Leu 305 310 315
- Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu Arg Val Lys Phe 325 330 335
- Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu 340 345 350
- Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp 355 360 365

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Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Gly Lys
Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
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| tgg gtg cag agc cag cag aag gtg cag cag agc cca gaa tcc ctc agt Trp Val Gln Ser Gln Gln Lys Val Gln Gln Ser Pro Glu Ser Leu Ser 20 25 30 | 96 |

| Val | cca Pro | gag Glu 35 | gga Gly | ggc Gly | atg Met | gcc Ala | tct Ser 40 | ctc Leu | aac Asn | tgc Cys | act Thr | tca Ser 45 | agt Ser | gat Asp | cgc Arg | 144 |
|--|--|---|--------------------------------|-------------------------------|--------------------------------|----------------|--------------------------------|-------------------------|-------------------------|-------------------|----------------|--------------------------------|-------------------------|-------------------|--------------------|-----|
| | | | | | | | | | | | | | | ggc Gly | | 192 |
| | | | | | | | | | | | | | | ggc Gly | | 240 |
| | | | | | | | | | | | | | | cac His 95 | | 288 |
| | | | | | | | | | | | | | | gtt Val | | 336 |
| gat Asp | tat Tyr | aac Asn 115 | cag Gln | GJ À aaa | aag Lys | ctt Leu | atc Ile 120 | ttt Phe | gjå aaa | cag Gln | ggt Gly | acc Thr 125 | aag Lys | tta Leu | tct Ser | 384 |
| | aag Lys 130 | | | | | | | | | | | | | | | 393 |
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                             40
Tyr Met Tyr Trp Tyr Arg Gln Asp Thr Gly His Gly Leu Arg Leu Ile
His Tyr Ser Tyr Val Ala Asp Ser Thr Glu Lys Gly Asp Ile Pro Asp
                     70
Gly Tyr Lys Ala Ser Arg Pro Ser Gln Glu Asn Phe Ser Leu Ile Leu
                                     90
Glu Leu Ala Ser Leu Ser Gln Ser Ala Val Tyr Phe Cys Ala Ser Ser
Asp Phe Ala Gly Thr Gly Gly Phe Tyr Glu Gln Tyr Phe Gly Pro Gly
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